STUDIES ON THERMOPHILIC BACTERIA

I. AEROBIC THERMOPHILIC BACTERIA FROM WATER[1]

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I. INTRODUCTION

The discovery of microörganisms that are able to live at relatively high temperatures (60°C. and above) has forced us to change our ideas on the resistance of protoplasm to heat and to admit that life is possible above the generally fixed limit of 42° to 45°C. The term thermophilic was probably first used by Miquel (1879) to describe those organisms that grow at temperatures so high as to be fatal to most microörganisms. This conception seems to have been lost sight of by many more recent workers. In order to have a better understanding of what the term thermophilic means, a number of definitions of the term as found in different texts on bacteriology are included.

In his physiological classification of bacteria, Giltner designated as thermophilic those that have a minimum temperature of 45°C., optimum, 55°C. and maximum, 70°C. Muir and Ritchie define thermophilic bacteria as organisms that grow best at a temperature of from 60° to 70°C. Hiss and Zinsser say that thermophilic bacteria are high temperature bacteria obtained from hot springs and from the upper layers of the soil. Rahn in Marshall's Microbiology describes thermophilic bacteria as extraordinary organisms having their maximum between 70° and 80°C., a temperature which coagulates albumin; corresponding to the high

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maximum the thermophiles have a very high optimum, and the minimum lies with most species above 30°C. According to Hewlett there is a group of so-called thermophilic bacteria which thrive best at a temperature of 60° to 70°C. Those bacteria whose optimum temperature is above 40°C. and which are spoken of as the "thermophil" bacteria, is the definition given for them by Morrey. Buchanan does not mention thermophiles in his book but speaks of the organisms which produce large quantities of heat as thermogenic bacteria. In his book Chester places thermophilic bacteria in a class that does not grow at room temperatures or below 22° to 25°C.

II. RELATION OF HIGH TEMPERATURES TO LIFE

All living things have their minimum, maximum and optimum temperatures for growth and other functions. The range of temperatures at which they exist may depend among other factors, on the species and on the ancestral history of the individual. Many investigators have experimented on the growth of organisms at high temperatures with varied and interesting results.

The first data concerning organisms that live at high temperatures were published by Sonnerat (1774). He reported on fish that lived in water at a temperature of 69°C. Schwabe (1837) reported the growth of algae in a hot spring at Karlsbad at 70°C. Flourens in 1846 mentioned algae which flourished in a hot spring at a temperature of 98°C. Brewer (1866) found some "Nostocformen" in a hot geyser at 83°C. Ehrenberg reported the existence of red and green algae from the Island of Ischia which grew at 63° to 65°C.

III. HISTORICAL

The literature on thermophilic microörganisms has already become quite voluminous and in order to save space in this publication, we have summarized in table 1, those papers which a careful search of the literature has revealed.

IV. EXPERIMENTAL

Sources of cultures. The present investigation has been limited to a study of some of the characteristics of 52 cultures of aerobic thermophilic bacteria from water; a more detailed study of forms from other sources is now being made. The cultures of thermophilic bacteria used in this investigation were isolated from samples of water furnished by the Illinois State Water Survey. Permission to use the samples was obtained from Professor E. Bartow, while director of the Water Survey. These samples came from different places in the state of Illinois and had been collected and shipped to the Water Survey according to directions furnished by them.

Many types of waters from different sources including deep wells, shallow wells, drilled wells, dug wells, springs, raw and treated municipal supplies and springs were used. In this manner it was possible to carry on a more representative study than if the samples had been taken from a restricted area. Out of 224 samples of water, 60 were found to contain thermophilic bacteria according to the method adopted for their isolation. This is to be regarded as a minimum for it is believed that thermophiles are quite abundant in nature and many samples which were negative when 1-cc. portions were examined would probably have been positive had a larger amount been used.

Method of isolation. Agar plates were poured in the usual way using 1-cc. and 0.1-cc. portions of the samples; the plates were incubated at 55°C. for twenty-four hours. Most of the thermophiles grow very rapidly at 55°C. and a longer incubation period was unnecessary. Any colonies that had developed in that length of time were transferred to agar slants; later it was found that it was easier to keep the cultures in broth since agar dried so quickly at 55°C. Control plates and agar plates that had been exposed to the air of the laboratory were incubated under the same conditions, but in no case was there any growth of thermophiles shown, either from the agar itself or from the air of the laboratory.

TABLE 1

INVESTIGATOR	ORGANISMS DESCRIBED	SOURCE	TEMPERATURE	REMARKS
Miquel (1879–88)	*Bacillus thermophilus*	Seine River water; sewage excreta; dust; air	42°–70°C. 65°–70°C. optimum	Attributes the property of this organism to grow at such high temperature to particular character of protoplasm. Isolated first in 1879; characteristics described in 1888
Van Tieghem (1881)	(1) Streptococcus (2) Bacillus	Water in which beans had been cooked	Up to 74°C. Up to 77°C	A certain amount of acidity produced by these organisms soon rendered the media uninhabitable by them
Certes and Garrigou (1886)	(1) Small rods (2) Filaments	Hot spring at Luchon	45°–64°C.	"Further experiments are necessary to determine the chemical and biological action of these rods, and this knowledge will throw a light on the therapeutics of mineral waters"
Globig (1888)	Many bacilli (30 kinds)	Garden soil	50°–70°C.	Thought the fact that most of them were isolated from the superficial layers of the soil explains that they get heat for high temperature at which they grow from the rays of the sun. Source not intestinal tract of man or animals, tap or river water
Burrill (1889)	Two bacilli	Silage; manure	60°–70°C.	The initial high temperature which these bacteria induce is probably most serviceable by causing the closer packing of the silage and the exclusion of the air, rather than by killing the germs of other ferments
Schloessing (1889)		Stable manure	Up to 79.5°C.	At temperature of 60°–66°C. these organisms produce 17 times as much carbonic acid as that in sterilized manure
Cohn (1893)				Attributed to thermogenic bacteria a rôle in so-called spontaneous heating of malt, tobacco leaves, cotton, hay, and manure. Made no attempt to isolate any of these cultures by the plate method

Flügge (1894)	Many bacteria	Sterile milk	24°–44°C. or 27°–54°C.	All strongly peptonizing in character; some were toxic; all formed spores which would withstand heating in water or steam for two hours
Leichman (1894)	A bacillus	Slimy milk	45°–50°C.	Produced acid
MacFadyen and Blaxall (1894)	Many bacilli	Earth, river and sea water, river mud, air dust, straw and feces of men, mice and chickens	60°–65°C.	"Their most marked property appears to be the decomposition of proteid bodies which they are able to effect." Most of them possess active fermentation properties
Gorini (1895)		Milk	37°–	Ambros (1910) suggested that this organism was thermotolerant since it grew at 37°C. also
Karlinski (1895)	(1) *Bacillus Illidzensis capsulatus* (2) *Bacterium Ludwigi*	Hot springs of Illidze in Bosnia	50°–58°C. 55°–57°C.	Has no explanation to offer as to significance of the presence of these two organisms in hot springs. Suggests that the examination of other hot springs for similar bacteria would greatly increase the knowledge of biology of water bacteria
Rabinowitsch (1895)	Eight species of thermophilic bacteria	Many sources; widely distributed in nature	34°–75°C.	Concluded that peculiar ability of so-called thermophilic bacteria to grow at temperatures so much higher than the optimum temperature for common bacteria is a property of adaptation to environment
Weber (1895)	Bacillus I Bacillus II Bacillus III	"Sterile" milk	22°–60°C. 22°–60°C. 30°–65°C.	Found thermophilic bacteria in eight out of eleven samples of so-called "sterile" milk. Two of the three bacilli formed spores; none liquefied gelatin; all produced hydrogen sulphide rapidly
Harding (1896)	Two or three kinds of thermophilic bacteria	Silage	50°–60°C.	No attempts made to characterise or identify the forms found

TABLE 1—*Continued*

INVESTIGATOR	ORGANISMS DESCRIBED	SOURCE	TEMPERATURE	REMARKS
Kedzoir (1896)	Cladothrix form	River Spree	36°–70°C.	Facultative anaerobe grows better without oxygen. Spores very resistant to heat and to disinfectants such as 5 per cent phenol
Teich (1896)	Bacillus form	Hot spring of Illidze	54°–58°C.	Large oval spores formed in one end of the rods make them appear club-shaped
Davis (1897)	Probably a bacillus	Hot springs of Yellowstone Park	Up to 85°C.	Functions in the formation of mineral deposits in hot springs
Harshbarger (1897)	White filamentous bacteria	Hot springs of Yellowstone Park	85.4°C.	Becomes of a sulphur yellow color at 175°F. Yellow growth due to species of Beggiatoa, a plant which is classed with the Bacteriaceae, and which, during life, deposits sulphur granules
Koning (1897)	*B. tabaci* III *B. tabaci* IV *B. tabaci* V	Tobacco		Ambroz (1910) speaks of these organisms as real thermophilic bacteria
Miyoshi (1897)	Many bacillus forms; a zooglian mass of bacteria; iron bacterium	Hot springs in Japan	41°–69.8°C.	Hot springs of Japan furnish a good medium for thermophilic bacteria
Wittlins (1897)		Hot springs in Switzerland		Ambroz (1910) explains the negative results of Wittlins on the grounds of inadequate methods of research
Laxa (1898)	*Clostridium gelatinosum*	Füllmasse in sugar manufacture	25°–58°C.	This organism is a facultative anaerobe whose spores are not killed by exposure to steam at 100°C. for 15 minutes

Oprescu (1898)	*Bacillus thermophilus liquefaciens aerophilus*	Soil, Berlin Zoological gardens	21°-70°C.	Oprescu gave rather a detailed description of the five forms studied by him
	Bacillus thermophilus aerobius	Canal water	36°-60°C.	
	Bacillus thermophilus aquatilis	Spring water, ice	36°-60°C.	
	Bacillus thermophilus reducens	Blood serum test glass	36°-62°C.	
	Bacillus thermophilus liquefaciens tyrogenus	Roquefort cheese	Room temperature to 60°C.	
Poupe (1898)	An organism similar to *Clostridium gelatinosum Laxa* (1898)	Syrup	45°C.	
Schillinger (1898)	Four types	Soil	66°C. max.	After experiments on bacteria from soil carried on at different temperatures, he came to the conclusion that thermophilic bacteria are not properly so-called; the term thermotolerant should be applied to those organisms which can adapt themselves to high temperatures
Tsiklinsky (1898 and 1899)	*Thermoactinomyces vulgaris* *Thermomyces lanuginosus*	Soil	48°-68°C.	Also isolated 6 varieties of bacteria from the hot springs on Island of Ischia which she called strict thermophiles; optimum temperature 60°C.
	B. thermophilus aquatilis liquefaciens *B. thermophilus liquefaciens aerobius*	Spring water, Berlin	50°-60°C. optimum	Michaelis said of these organisms that they were not only thermotolerant, but also thermophilic
Michaelis (1899)	*B. thermophilus aquatilis chromogenes* *B. thermophilus aquatilis anguinosus*			
Vernhout (1899)	*Bacillus tabaci fermentationis*	Fermenting tobacco at 44°-50°C.	25°C. optimum	Not a true thermophile since its optimum temperature was 25°C.
Sames (1900)	Nine organisms not named	Water, feces, soil, pus, milk, etc.	50°-70°C. optimum	Sames suggests that a distinction be made between "thermophilic" and "thermotolerant" bacteria

TABLE 1—*Continued*

INVESTIGATOR	ORGANISMS DESCRIBED	SOURCE	TEMPERATURE	REMARKS
Dupont (1902)	*Bacillus thermophilus* Grignoni	Manure	50°C.	Also found *B. subtilis* and *B. mesentericus ruber* a few times at this high temperature. The organism was proteolytic in character
Russell and Hastings (1902)	A micrococcus	Pasteurized milk	20°–25°C. optimum 76°C. maximum	Not a true thermophile but probably a thermotolerant organism
Schardinger (1903)	Group I—6 varieties Group II—1 aerobic and 5 anaerobic organisms	Foods, etc. Foods, etc.	Room—35°C. 37°–66°C.	Grouped with hay and potato bacilli. Produce NH_3 from nitrates. Found that these bacteria "dextrinize" starch
Setchell (1903)	Filamentous Schizomycete	Hot springs of Yellowstone Park	70°–89°C.	Such bacteria grow at a higher temperature in silicious than in calcareous waters
Tsiklinsky (1903)	Eighteen bacilli. Two streptothrix forms	Human alimentary tract	57°C.	Believed the constant appearance of thermophilic bacteria in feces was explained by their wide distribution in nature and their great resistance; thermophiles are probably merely variations of common non-thermophilic organisms
Catterina (1904)	*Bacterium thermophilus radiatus*	Water	60°–70°C. optimum	Also isolated an organism apparently identical with *B. thermophilus* IV (Rabnowitsch, 1895)
Gilbert (1904)	*Actinomyces thermophilus*	Soil	50°–55°C. optimum	Strict aerobe; liquefied gelatin slowly; coagulated milk
Kehler (1904)	Two forms		65°C. optimum	
Benignetti (1905)	A bacterium	Hot spring	60°–75°C. optimum	An anaerobic gram positive organism which produced large central spores
Bruini (1905)	Thirteen bacilli, five streptothrix forms	Adult and infant stools		Four bacilli and one streptothrix absolute thermophiles; all strict aerobes; all gram positive; all but one spore formers

Miehe (1905)	*Bacillus thermophilus* alpha	Hay	40°-70°C.	Hay in which spontaneous combustion had occurred
Anitschkow (1906)			59°-60°C.	Only occasionally found thermophiles in human alimentary tract
Bardou (1906)	*Bacillus thermophilus* alpha *Bacillus thermophilus* beta *Bacillus thermophilus* gamma *Bacillus thermophilus* delta	Sewage	50°-60°C. optimum	Ambros says Bardou's work on the chemical reactions of thermophiles is fundamental and far surpassed in detail and accuracy other work that had been done. Three of organisms described were denitrifiers; all of them were strongly proteolytic
Blau (1906)	*B. cylindricus* *B. robustus* *B. tostus* *B. calidus*	Soil	60°C. optimum	Thermal death point 100°C. for 20 hours Thermal death point 100°C. for 7½-8 hours Extremely resistant spores. Thermal death point 100°C. for 19-20 hours Thermal death point 100°C. for 8 hours
Brazolla (1906)				Regarded thermophilic bacteria of sanitary significance in water
Falcioni (1907)	*B. thermophilus* I *B. thermophilus* II *B. thermophilus* III	Hot springs	60°C. optimum	Concluded that hot springs form favorable media for thermophilic bacteria
Miehe (1907)	*B. calfactor*	Hay	50°-60°C. optimum	Claimed this organism was responsible for heating of hay. Called an orthothermophile. (Maximum above coagulation point of protein, 60°-70°C.)
Tirelli (1907)	Four rods; four cocci; two thread forms	Drinking water	55°-65°C. optimum	Believed temperature relations due to particular chemical nature of protoplasm rather than to their adjustment to the circumstances of their environment
Schütze (1908)	Many forms of *B. calfactor* (Miehe) type	Moist clover hay		Believed that thermophiles grew better under aerobic conditions at high temperature and at lower temperature they grew better under anaerobic conditions

TABLE 1—*Concluded*

INVESTIGATOR	ORGANISMS DESCRIBED	SOURCE	TEMPERATURE	REMARKS
Jäger (1909)				Discussed possibility of spontaneous combustion of different organic materials by thermophilic bacteria
Ambros (1910)				A complete review of literature on thermophiles up to 1910
de Kruyff (1910)	Ten rod forms	Soil, water, air in tropics	55°–65° C.	Claimed thermophiles were very abundant in tropical climates Proved thermophiles were very important in biological changes in nature
Georgevitch (1910)	*B. thermophilus* Vranzensis	Hot springs	56°–60°C. optimum	
	B. thermophilus Jivioni		43°–45°C. optimum	
	B. thermophilus Losanitche		72°–73°C. optimum	
Koch & Hoffman (1911)	Two spore-forming bacilli One spore-forming bacterium One thread form	Soil	52°C.	Concluded that the nature of the media had a great influence on the temperature response of these organisms, since they grew in soil at a temperature of 28°–30°C. and would not grow on artificial media at this temperature
Barlow (1912)		Canned corn		An organism said to be responsible for spoilage of canned corn
Kroulik (1912)				Thought bacteria and actinomyces forms which decompose cellulose at 60°–65°C. are widely distributed in nature and occur specially where cellulose is naturally decomposed
Noack (1912)				Review of literature
Ambros (1913)	*Denitrobacterium thermophilum*	Soil	37°C. below minimum temperature	Called a true thermophile because it would not grow at room temperature nor at 37°C. Ambros thinks the importance of thermophilic bacteria in the cycles in nature can not be overestimated since they play such an important rôle in metabolic processes

Negre (1913)	Numerous forms	Sand of Sahara		
Pringsheim (1913)				Studied decomposition of cellulose by thermophilic bacteria
Kossowicz (1912–13)				Studied thermophilic bacteria in sugar juices
Bergey (1919)	Nine different bacteria	Dust, soil, etc.	60°–80°C. maximum	Suggested separating into two groups, the true thermophiles and the facultative thermophiles
Cheney (1919)	Organisms like I, VI, and VI of Rabinowitsch	Canned foods	55°C.	Explained fact that so few thermophiles are found in canned foods on the market by supposing that nearly all cans containing them become spoiled and so are automatically eliminated before reaching the market
Patzschke (1919)	*Streptococcus lacticus thermophilus*	Milk		
Weinzirl (1919)	*B. thermoindifferens* *B. aerothermophilus* *B. thermoalimentophilus*	Canned foods	55°C. optimum 55°C. optimum 55°C. optimum	
Donk (1920)	*B. stearothermophilus*	Canned corn; string beans; corn on cob	50°C. optimum	Found in corn that had been processed at 118°C. for 75 minutes
Bigelow and Esty (1920)				Studied the thermo-resistance of spores of thermophilic bacteria. Found that the hydrogen ion concentration of the media had a definite influence. Used term "obligate thermophiles"
Grieg-Smith (1920)	Spore-bearing rod	Fermenting tan-bark	60°C. optimum	May live at 80°C. in the fermenting tan-bark stacks

Methods of study. Inoculations into the different media used in this work were made either from twenty-four-hour agar slant cultures or twenty-four-hour broth cultures. Since this work was begun before the adoption of the new chart, the Descriptive Chart of the Society of American Bacteriologists indorsed in 1914 was used in the study of these thermophiles. The group number for each culture was determined under as uniform conditions as possible. In the work that is now under progress on thermophilic bacteria from soil, canned foods, and other sources the Descriptive Chart indorsed by the Society of American Bacteriologists at the meeting of December 30, 1920, will be used. The index number, it is believed, will give a better description of the organisms since it seems to embody characteristics which are more important.

Media and technic. With one or two exceptions the media and technic used in this study were those recommended by the Committee on the Descriptive Chart of the Society of American Bacteriologists in their report on Methods of Pure Culture Study (1920); the cultures were, however, all grown at 55°C. Other exceptions will be mentioned later.

Morphology. All the cultures studied were motile rods and usually grew in chains containing from two to many individuals. Sometimes chains of four or five rods showed an active snake-like movement. The rods were both long and short; some had rounded ends. Carbol fuchsin and Gram stains were used to stain the smears; all were Gram positive except nos. 10, 20, 40. Without exception, the cultures studied formed spores. Some of the spores were central and some polar; some were oval and some round; in a few cases the diameter of the spore seemed to be larger than that of the rod and produced a sort of clostridium form.

Nutrient broth. Witte's peptone was used in the nutrient broth employed in this study, since it seemed to possess certain advantages over other peptones. Good growth was secured with all strains in nutrient broth at 55°C. Most of the cultures produced turbidity and sediment in the broth; the surface growth in many of the cultures was membranous or showed a heavy pellicle.

Indol. Tests for indol were made on nutrient broth cultures that had been incubated for four days at 55°C. Both Ehrlich's test and the Nitroso-indol test were used. It was found that Ehrlich's test was much more satisfactory when the tubes were heated slightly. All of the cultures formed indol from Witte's peptone in varying amounts.

Hydrogen sulfide formation. To determine hydrogen sulfide formation, nutrient broth (made of Witte's peptone) over which a strip of lead acetate paper was suspended by means of the cotton plug, was used. The cultures were incubated for four days at 55°C. The blackening of the paper indicated hydrogen sulfide formation. All the cultures studied formed H_2S. Streak cultures on "Bacto Lead Acetate Agar" plates also showed that all the cultures formed H_2S. This latter medium seemed to be well adapted to the determination of this characteristic.

Potato slants. The growth of these thermophiles on potato slants was abundant in most cases even after twenty-four hours at 55°C. The type of growth varied from a filiform to spreading growth. The potato was turned gray, brown, or reddish brown. The cultures could not be kept longer than from two to three days since they dried out so quickly at 55°C., but the growth at this temperature was quite rapid on this and other media.

Liquefaction of gelatin. The "provisional method" was used to determine this characteristic. The cultures were first accustomed to the gelatin medium by preliminary cultivation for eighteen to twenty-four hours in a 1 per cent solution of gelatin at 55°C.; then the surface of gelatin in test tubes was inoculated and the tubes incubated for thirty days at 20°C. All the cultures except nos. 6, 15, 20, 32, 51 had partially or completely liquefied the gelatin at the end of thirty days. Gelatin cultures prepared in the same way and incubated at 55°C. for four days were all liquefied with flocculent growth throughout the gelatin and would not harden when placed in the refrigerator. The fact that all the cultures studied liquefied gelatin at 55°C. and some of them at 20°C. can probably be explained by the fact that 20°C. was below the minimum temperature for growth for those cultures which did not liquefy gelatin at that temperature.

Litmus milk. In this medium azolitmin was used as the indicator. The litmus milk cultures were incubated at 55°C. for four to seven days, only, because they evaporated so quickly at this temperature. Peptonization occurred with at least 75 per cent of the cultures; and in each of these cases the medium was alkaline. All of the cultures curdled the milk and in those cases where peptonization did not occur the medium was acid. More work is being done on the growth of these organisms in milk and particularly on the use of brom-cresol purple as an indicator.

Fermentation of sugars and glycerol. No gas was formed by any of the cultures. None of the cultures produced acid in lactose; the cultures varied slightly in their formation of acid in glucose, sucrose and glycerol broth. Brom thymol blue was used to test for acidity since that indicator was used to adjust the reaction of all the broths when made. The amount of acid formed in the different broths by these cultures may be of significance and should be determined.

Oxygen relation. This characteristic was determined by noting the presence or absence of growth in the open and closed arm, respectively, of fermentation tubes containing glucose broth. All the cultures used in this study were found to be strict aerobes.

Reduction of nitrates. To determine this characteristic, both nitrate broth and nitrate agar slant cultures incubated at 55°C. for four days were used. Sulphanilic acid and alphanaphthylamine were used to test for nitrites. All of the cultures reduced nitrates.

Diastatic action on starch. Two per cent agar containing 0.2 per cent of soluble starch was used for this determination because this stiffer agar seemed to stand the incubation at 55°C. better. Dot inoculations were made in the center of the petri dishes containing the hardened starch agar; these were incubated at 55°C. for forty-eight hours since longer incubation dried the agar and made it crack. All the cultures grew well on this media and all produced diastatic action, some feeble and some strong.

Temperature relations. Some of the cultures were grown on agar slants at different temperatures and it was found that 50° to 55°C. was the optimum temperature for growth. Since it is believed that the temperature relations of the thermophilic group of microörganisms is a subject worthy of intensive study, a separate investigation on this subject was initiated which is now nearing completion in this laboratory.

V. DISCUSSION

A comparative study of 52 strains of thermophilic bacteria from water indicates a group in which the characteristics are not widely divergent. All of the strains were spore formers and all liquefied gelatin. When separated into groups according to the "group number" they fell into nine groups. Most of these groups were defined by differences in the terminal reaction in glycerol and carbohydrate media. If these determinations are neglected all of the strains would have fallen into one group.

A survey of the literature on thermophilic bacteria indicates that many of the strains there described have been superficially studied and that new strains have been named without sufficient data. Consequently many of the names which are used for thermophilic bacteria are being applied to the same organism.

Without exception the 52 cultures which were used in this study formed spores and in this characteristic seem to agree with most of thermophilic bacteria which have been described in the literature. This then seems to be the most common characteristic of members of this group. It has also been the basis for including among the thermophilic bacteria, bacteria which do not belong there. Spore formation when taken into consideration along with the peculiar reaction to temperature makes the thermophilic bacteria a difficult group for canners of foods, for instance, to cope with. The ability to form spores allows the thermophilic bacteria to survive the process and perhaps to develop when the cans are stacked in the warehouse. The recent publications of Weinzirl, Cheney, Bigelow and Esty, and others have indicated the significance of these bacteria. They are also related to certain phases of the dairy industry. Flügge, Leichmann, Russell and Hastings have found that they are able to survive pasteurization temperatures.

The forms isolated from water were Gram positive and in this characteristic, also, agreed with the forms described in the literature. Their destructive nature is indicated by their action on the proteins in milk and on gelatin. Indol and hydrogen sulphide were produced in most media. Rabinowitsch (1895) reported the proteolytic abilities of the thermophiles to be their most characteristic function.

Although none of the cultures studied fermented any of the sugars used, many of them did produce some acid in glucose or sucrose broth; this together with the fact that all of them showed diastatic action on starch would seem to indicate that these thermophiles decompose the more complex carbohydrate molecules more readily than they do the simple sugars. That many of the thermophiles decompose cellulose quite readily is seen in a review of the literature on those types that function in spontaneous heating during the fermentation of malt, tobacco, cotton, hay, and manure, the fermentation of silage and the decomposition of cellulose.

The fact that some of these cultures came from water from quite deep wells and others from surface waters demonstrates that even in water the thermophiles can exist at widely varying ranges of temperature. They are probably widely distributed in waters and had larger portions of the samples of water been used for plating, no doubt, many more cultures would have been isolated from the 224 samples of water examined. The widely differing sources of the thermophiles described in the literature also indicate that they are widely distributed in nature and further investigations of their temperature relations will explain this distribution. Rabinowitsch (1895) and some others have claimed that this ability of thermophiles to grow at a high temperature was a property of adaptation to environment. Blau and Bruini both claimed that many non-thermophiles had many of the same characteristics as thermophiles. Other investigators have published data to support this claim. Bredfeld (1878) gradually developed the resistance of spores of *Bacillus subtilis* until it took three hours at 100°C. to kill them or five minutes at 110°C. Koch (1876) observed the germination of spores of *Bacillus* *anthracis* and *Bacillus subtilis* that had been subjected to 123°C. in dry air. According to Arloing, Cornevin, and Thomas (1882) the spores of *Bacillus anthracis-symptomatici* would not resist boiling for more than 2 minutes; but if previously dried, boiling for two hours was necessary to destroy them.

This seems to agree with the theory of Davenport and Castle (1895) that by the loss of water, which is a necessary consequence of increased chemical activity resulting from warmth, organisms are able to increase their resistance to high temperatures. If we accept this view thermophiles are explained on the basis of adaptation to environment.

Tsiklinsky (1903) also believed that thermophiles were merely variations of common non-thermophilic microörganisms that had adapted themselves slowly to high temperature. He thought that the length of time necessary for these organisms to adapt themselves to high temperature determined whether they were facultative or strict thermophiles.

Schillinger proposed the term thermotolerant to be applied to these organisms. Miehe tried to explain the high optimum temperature of thermophiles by assuming that they might have been brought over from the tropics and have adapted themselves to lower temperatures. By comparing them with other bacteria, Miehe came to the conclusion that all bacteria could be grouped on the basis of their minimum temperatures. He divided the thermophiles into two groups: (1) orthothermophiles with a maximum temperature of 60°–70°C.; (2) thermotolerants with a maximum temperature of 50°–55°C. but which also grow well at ordinary temperatures.

Many investigators, among them Rabinowitsch, Schütze, and others, found a certain parallelism between temperature relations and the relation of thermophiles to oxygen. In most cases those organisms which had high optimum temperatures were strict aerobes.

Bergey divided thermophilic bacteria into two groups: (1) true thermophiles, those that grow at temperatures above the maximum temperature for the great majority of bacteria, especially the pathogenic forms; (2) facultative thermophilic bacteria, those that develop at room temperature, about 20°C., and have their optimum temperature at about 50°C., and their maximum temperature at about 60°C.

It would seem to be indispensable to fix clearly the limits within which the term thermophilic bacteria should apply. Some division evidently must be made in this group of organisms that grow at such widely differing ranges of temperature. The division made by Bergey into true thermophiles and facultative thermophiles seems to be the most tenable up to the present time. Further work on temperature relations of these thermophiles is being carried out in this laboratory and perhaps when the data from this investigation are available a better differentiation will be possible.

Of the 52 waters from which thermophilic bacteria were isolated 44 (almost 85 per cent) were condemned for the presence of *B. coli* of fecal origin. This fact suggests a possible sanitary significance of thermophilic bacteria in water analysis. The data are insufficient to draw any definite conclusions on this subject but it is a subject worthy of investigation. A similar suggestion was made by Brazzola (1906) when he stated that he thought the thermophiles were of very great importance in the study of the potability of water.

VI. CONCLUSIONS

1. The aerobic thermophilic bacteria studied in this investigation seemed to make up a closely related group when the salient characters only are considered.
2. All strains form spores and are strongly proteolytic which, in connection with their temperature relations, makes them of importance in food preservation.
3. Thermophilic bacteria are widely distributed in nature (soil, water, etc.) and thus may cause serious losses in those industries where high temperatures are used for controlling bacterial development.
4. The ability of thermophilic bacteria to grow at high temperatures may be due to a particular property of the protoplasm (water content?).

5. Further investigations on their temperature relations may aid in a better understanding of the thermophilic bacteria and in their separation into more sharply defined groups. This work is in progress in these laboratories.

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